was extensively investigation, no gametes carrying a new modification of it that alters its capacity of action have yet been identified. However, in a few plants and kernels having it, sectors showing the Spm-s capacity have appeared but their frequency is low. In contrast, another Spm-w isolate exhibits relatively frequent returns to or twoard the Spm-s type capacity of action.

e). Transposition of Spm

Initially, the most compelling evidence of unit elements, independent of the gene but capable of controlling its action, came from discovery of their transposition from one location to another in the chromosome complement without los~~ing~~s of specificity of action in the process. Transposition is a behavioral mechanism that all of them may share in common. However, in studies of this, it was learned that an element known to be transposible, may not undergo transposition within a given set of conditions. When these conditions apply, it could be difficult to determine whether or not the observed altered phenotypes were the result of action of a controlling element, ~~or~~ of gene mutation, ~~or~~ of the presence of a modifier or because of a mutator-gene. In the cases we have examined, this could be distinguished on the basis of known histories of origin and of of comparitive behaviors under given conditions. In other words, transposition makes possible ready identification of a controlling element but lack of evidence of this does not exclude, necessarily, the possibility that a controlling element is responsible for observed types of mutation or of instability of gene ~~action~~ expression. There is evidence to suggest that controlling elements in maize may play a larger part in the origin of mutants than has previously been suspected, and this will be considered in the concluding section of this report.

Evidence of transposition of Spm is extensive. It has been observed in various types of experiment conducted for purposes other than that of examining the phenomenon of transposition itself. Tests conducted for the latter purpose reveal more about it than evidence, however, extensive, obtained from other types of tests. Therefore, in this report, only those tests will be reported in detail that are centered on an examination of transposition of Spm.

There is no doubt that the time during development when it occurs, and also ~~and~~ the frequency ~~of occurrence of~~ of this transposition of Spm are under some form of control, just ~~such~~ as the time and frequency of occurrence of mutation at $a_1^{m-1}$ and $a_2^{m-1}$ are controlled by the state of one of them that is present in any one plant or kernel. Up to now, the conditions responsible for ~~such~~ control have not been ~~identified~~ defined and no tests have been conducted that might serve to reveal them, although such are possible. Nevertheless, it is suspected that ~~the~~ such control may reside in the Spm element itself, and that changes in this may reflect some alteration that occurred to this element. Even though an examination of factors associated with control of transposition of Spm were not investigated ~~neglected~~, the following types of control are known. With some isolates of Spm, transposition of it occurs in some cells early in plant development. With others, this occurs only late in development and with still others, transpositions may occur both early and late in development. With ~~still~~ other isolates, transposition of Spm, either early or late, may occur only very rarely, and with one isolate, no evidence of transposition has yet been obtained although it has received extensive study.

~~The~~ Spm ~~system~~ is not well suited ~~ideal~~ for study of ~~examining~~ the mechanism associated with transposition. This is more readily accomplished with other systems, and that of Ds, or of Ac at the P or $Bz_1$ loci, serve this purpose better. No additional evidence of the mechanism of transposition ~~involved~~ has come from this study of Spm.

f). Modifier element in the Spm system

In the course of study of $a_1^{m-1}$, a series of tests were being conducted to determine Spm number and location in nearly a thousand (995) plants, all of which were derived from the second generation backcross to plants that were homozygous for the standard $a_1$ locus and in which no Spm was present. The state of $a_1^{m-1}$ was the same in all plants that carried it. The behavior of this state with a fully active Spm is shown in photograph With ~~this state~~ it, mutations occur late in development of both plant and kernel, ~~with~~ there being only a few exceptions. to this ~~For this reason~~ Consequently, it would be expected to ~~be~~ remain stable in expression ~~in~~ through successive plant generations, and this was found to be true. In fact, although it has been used very extensively in these studies, no ~~gamete~~ kernel or plant carrying a newly altered state of it has ~~appeared~~ been recognized in any test. However, ~~a~~ one of the plants in the test series mentioned above that was $a_1^{m-1}/a_1$ and carried one Spm element in chromosome 5, was crossed by one that was homozygous for $a_1$ and had no Spm. On the resulting ear, ~~xxxxxx~~ all $a_1^{m-1}$ carrying kernels that also had Spm exhibited the expected pattern of mutation to $A_1$ ~~with~~ that this state produces with the exception of one kernel. This exceptional kernel exhibited a marke~~dly~~ increased number of $A_1$ mutant spots in comparison with that shown by the other variegated kernels on the same ear, (~~and this is~~ see illustrated ~~in~~ photographs ). Its phenotype suggested that an alteration of state of $a_1^{m-1}$ had occurred in an

of

ancestor cell t~~hat produced~~ this kernel. The plant derived from this kernel likewise exhibited tha same marked increase in frequency of mutation. Tests were conducted with this plant in order to examine the nature of the altered phenotype, and these indicated that the increased mutation rate was not the consequence of an altered state of $a_1^{m-1}$ but rather of the presence of an independently located heritable unit. The effect on $a_1^{m-1}$ mutation rates produced by this heritable unit could be seen only when an active Spm element was also present in the chromosome complement. In the absence of Spm, or when it is inactive, no evidence of the present of this Modifier is noted. Tests of it conducted in subsequent plant generations indicated that it underwent frequent transposition and thus, could be considered as an independent controlling element within the Spm system. Its presence in any one kernel or plant could be detected readily by the increase in rate of mutation to $A_1$ it effects with those states of $a_1^{m-1}$ that are characterized by the production of relatively few such mutations in its absence. This rate

To that normally given by the

of increase of mutation is proportional ~~with~~ ~~eac~~h state in that the

over this mutations

number of mutant spots is increased by a factor somwhere between 2 and 3.

however,

The time when mutations occur is not altered by this element. Also, on states of $a_1^{m-1}$ that give very many mutations to $A_1$ in the presence of active Spm, this element appears to exert little or no effect. ~~Also,~~ There is no evidence to indicate that increased doses of it will effect a proportional increase in rate of mutation.

Tests of the action of the Modifier on four states of $a_1^{m-1}$ were conducted. In addition, tests of its transposition were made. Likewise, its behavior was examined with a fully active Spm-s element, an Spm-w element and also ones that are undergoing frequent change in phase of activity. It is of interest to note that it produces the same phenotype with either Spm-s or Spm-w.

No more is known of the origin of the Modifier other than that stated above, nor has another instance of origin of it been noted. It could be related both to the Spm element, since it enhances Spm-w activity, and to the element at $a_1^{m-1}$, since it exhibits one of the properties of this element, that is, control of rateof mutation to $A_1$.

g). Resume of mode of operation of the Spm system

From the descroptions given in the preceeding parts of this section, the reader may have gained the impression that the Spm system of control of gene action is inordinatly complex. There is no doubt that the analysis of a system of this type may be inordinately frustrating because of the many ~~xxxxxxxx~~ diverse phenotypes that may appear, even in different parts of an individual plant or in different areas of ~~one~~ a kernel, and also because of the irregular and ~~superficially~~ presumably irreconcilable ratios of phenotypes that may appear in progeny of sister plants or even in progeny derived from different parts of the same plant. It was because of this that study of $a_2^{m-1}$ had to be discontinued for a period of time, as no ~~effective~~ adequate interpretations came ~~was drawn~~ from observations and tests of it. Study of $a_1^{m-1}$ progressed more rapidly and successfully; and relatively early in its study, interpretations could be drawn of the basic mode of operation of the elements involved in ~~this~~ the system, and these have remained essentially valid for all observations and tests that have subsequently been conducted.

For a ready appreciation of the mode of ~~action~~ operation of this system, an understanding only of the two primary aspects are essential. The first is ~~xxxxxxxx~~ concerned with ~~an understanding of the~~ expressions given by each of the different states of $a_1^{m-1}$ and $a_2^{m-1}$, and the second is concerned with the basic mode of ~~control of this by~~ action of the independently located Spm element. All other aspects are secondary in that they are concerned almost exclusively with the degree of effective~~ness of~~ action of the Spm element at any one time, and the consequences of this with any one state. These secondary aspects, nevetheless, are responsible for much of the apparent ~~inordinate~~ complexity of this system, and for many of the difficulties and frustrations experienced in the course of its analysis.

As mentioned earlier, the states of $a_1^{m-1}$ and $a_2^{m-1}$ control the types of mutation, the time of their occurrence, and the frequency of this at any one stage in development when Spm is present and active. They also control the type of gene expression that appears in the absence of Spm. The states vary greatly with regard to these expressions. Thus, within the system, diverse phenotypes are produced that are attributible solely to state differences. ~~The primary action of~~ The Suppressor-mutator element, Spm, ~~is a~~ acts to suppress~~ion of~~ anthocyanin pigment formation in plant and kernel that appears with all but ~~expression of gene action, given by any~~ one state in its absence, and also to induc~~tion of~~ changes at ~~the~~ $a_1^{m-1}$ or $a_2^{m-1}$ ~~locus~~ that ~~lead to~~ result in altered ~~phenotypic~~ expressions of gene action. Included among them are mutations to stable alleles, either to a totally recessive, $a_1$ or $a_2$, or to higher alleles, each associated with the production of a particular type and with the degree of ~~production~~ this of anthocyanin pigment. ~~Another consequence of the~~ change~~s that~~ Spm may also induces a modification ~~of the~~ $a_1^{m-1}$ or $a_2^{m-1}$ that does ~~results~~ not give ~~in mutation to a~~

a stable mutant allele, but rather ~~to~~ an altered type of behavior of the locus in subsequent cell and plant generations both in the presence and absence of Spm, that is, to a change in its state.

## Part II. Origin and Behavior of $a_1^{m-1}$

### 1. Origin of $a_1^{m-1}$ and of its different states.

The origin of $a_1^{m-1}$ was outlined in the previous section. It first appeared in a single kernel on an ear produced by the cross of a plant homozygous for $a_1$ and for $A_2$ to one which was $A_1/a_1$ and $A_2/a_2$. The $a_2$ in this latter plant had been derived from mutation of $a_2^{m-1}$ to the stable recessive, $a_2$. All of the kernels on the ear, except one, were either uniformly dark pigmented ($A_1$ and $A_2$) or totally colorless ($A_2$, $a_1$). The exceptional kernel had spots of deep pigmentation in a colorless background. The plant derived from it (culture number 5371) likewise exhibited variegation for anthocyanin pigmentation. It had a number of distinct areas of deep pigmentation in a non-pigmented background. From the known constitutions of the parents, it was suspected that the instability of genic expression was associated with an alteration that had occurred at the $A_1$ locus in the heterozygous parent and in a single cell, late in development of the plant. Therefore the variegated plant was crossed with a number of plants that were homozygous for the standard recessive, $a_1$. It was also crossed with plants homozygous for the standard recessive, $a_2$, and to other homozygous for ~~other~~ recessive alleles of other genes known to be involved in development of anthocyanin pigment formation.

From these crosses it was readily determined that the variegated plant carried the standard recessive, $a_1$, in one chromosome 3, and a recently modified $A_1$ locus in the homologue, and that the modified locus was responsible for the expression of variegation. This locus was given the designation $\underline{a}_1^{\underline{m-1}}$. The original variegated plant, 5371, carried the standard $A_2$ in one chromosome 5 and a stable recessive, $a_2$, derived from mutation of $a_2^{m-1}$, in the homologue.

On the ears produced by the cross of the original $a_1^{m-1}$ plant to plants homozygous for $a_1$ and $A_2$, kernels exhibiting several different phenotypes appeared: those that were uniformly and deeply pigmented, those that were uniformly but lightly pigmented, those that had areas of deep and light pigmentation in a colorless background, and those that were totally colorless. ~~kernels~~. The types of pigmented areas and the patterns of these were much alike among nearly all of the variegated kernels. There were some large areas that were either deeply pigmented or more lightly pigmented and also many small areas with these pigment types, as illustrated in photo. . However, on a few ears, there were one or several kernels that exhibited a pattern of variegation that differed markedly from this. Some had only small spots of deep pigmentation in a colorless background, and the number of these ranged from only a few in some kernels to very many in others. Other exceptional kernels exhibited pigmented areas of various sizes in which the intensity was always low.

Kernels exhibiting different phenotypes were selected from some ears and planted in the greenhouse the following winter, 1950-51. The phenotypes of the selected kernels are entered in column 1 of table 1. The phenotype exhibited by each plant was similar to that expressed in the kernel from which each was derived. For example, if the kernel had shown only a few small spots of deep pigmentation in a colorless background, the plant likewise showed only a relatively few small streaks in which the deep anthocyanin pigmentation appeared. Or, a plant derived from a variegated kernel having pigmented areas whose intensity was low, likewise exhibited pigmented areas in which the grade of intensity of this was low. It was evident that the altered patterns of variegation in the selected kernels and in the plants derived from them arose as a consequence of some genetic modification controlling the time during development at which change in gene action occurs, ~~in~~ the numbers of cells in which such change occurs at any one time, and ~~in~~ types of pigmentation that would result from this. Confirmation of this precise type of control of gene action came from tests conducted with these plants, through self-pollination of them and through crosses of them to plants that were homozygous for the standard recessive, $a_1$. The phenotypes of the variegated kernels on the ears these crosses produced resembled that which had been expressed in the kernel from which each plant had arisen.

The kernels selected for greenhouse planting belonged to several different categories, enumerated in table 1. All four plants derived from kernls in row 1 carried a stable $A_1$ mutant of $a_1^{m-1}$. Those derived from the uniformly pale colored kernels in row 2 appeared to carry a stable intermediate allele of $A_1$ from tests conducted with them in the greenhouse. Subsequent tests conducted with their progeny indicated, that the $a_1^{m-1}$ locus in them was unmodified and that it would express the original type of variegation pattern in the presence of Spm. Tests of one of them, plant 5700A, and of its progeny will be considered in detail later. The two kernels in row 3 had been selected because each exhibited a few small colorless areas in what appeared to be a fully pigmented background. Tests conducted with them indicated~~, however~~, that this phenotype was not produced by somatically occurring change in gene action from $A_1$ to $a_1$ but rather it was produced by a very large number of late occurring mutations ~~from~~ to $A_1$.

of kernels that appeared on ears derived from self-pollination of these plants, and from crosses of them to plants that were homozygous for the standard recessive, $a_1$. The phenotypes of the variegated kernels in the progeny of ~~each of~~ any one of these plants resembled that which had been expressed in the kernel from which each had arisen.

(3) Tests of questionable plants [illegible]

¶ For the greenhouse planting, ~~a few~~ 2 kernels ~~were~~ had been selected ~~that appeared~~ because each ~~to~~ exhibited a few small colorless areas in what appeared to be a fully pigmented background (Row 3, [illegible]). Tests of the plants derived from them indicated, however, that this phenotype was not produced by somatically occurring change in gene expression from the $A_1$ type to $a_1$, but rather it ~~was~~ due to a very large number of late occurring mutations ~~from the $a_1$~~ to $A_1$. ~~When this occurs, large areas that appear to be uniformly pigmened may be present.~~ This may result in what appears to be a large fully pigmented area, but it arises from confluence of many small, closely placed areas each of which represents a ~~However, these do not arise from early occurring~~ mutation to $A_1$ ~~, but rather from confluence of pigmented areas~~. This confluence ~~does~~ is not ~~arise from~~ the consequence of direct contact of one mutant area with another but rather ~~from~~ of spread of some substance, produced in the cells of the $A_1$ mutant areas, ~~in~~to the surrounding genetically $a_1$ ~~areas~~ cells that allows pigment to be formed in ~~them.~~ the latter. This substance may spread through a number of cells, and it gives rise to a halo about each ~~genetically~~ $A_1$ mutant ~~area~~ spot, the intensity of pigment within the halo decreasing as the distance from the ~~mutant area~~ spot in which mutant cells are present increases. (If a pigmented spot appears in a large colorless area) Thus, whenever a large number of closely placed mutant

$A_1$ areas are formed, confluence of their many halos occurs. When this happens ~~occurs~~ within a large area of a plant or kernel, the area may appear to be uniformly pigmented and to have arisen from an early occurring mutation to $A_1$. However, microscopic examination of such areas usually reveals some irregularities within it in grades of intensity of pigmentation. This would be ~~Such is~~ expected ~~to appear from~~ if confluence of some halos occurs only at their border regions where pigment intensity is low ~~of halos of mutant areas~~.

Halos of the type described above do not surround ~~are not produced by mutant~~ areas exhibiting pigment intensities of lower grade. The borders of such mutant areas are sharply defined. Thus, a change in gene action at $a_1^{m-1}$ to full $A_1$- type expression may be distinguished from those that give the lesser intensities of pigmentation not only on the basis of the intensity of the pigment itself but also on the basis of presence or absence of halos ~~formation~~. It is suspected that the pigment produced in ~~the so-called~~ some distinct pale areas is not the same as that produced by the standard $A_1$ and some evidence of this will be given later. ~~This is shown more clearly in the plant than in the kernel~~. However, indirect evidence of it appears in plants. In them, the pale-~~producing~~ phenotype is expressed only in some tissues, and it is conspicuously absent from the leaf-blade except with the ~~in~~ the mid-rib. In contrast, the full $A_1$ phenotype is ~~readily~~ expressed in the leaf-blade, intense pigment being expressed within it. Also, in the plant, some of the pale-producing mutants give rise to anthocyanin pigments with pastel shades that obviously would not appear merely from reduction in amount of the same pigment that is produced ~~by~~ with the standard $A_1$. It is likely that the action of the pale mutants ~~produces~~ result in pigments that differ from that ~~appearing when~~ produced with standard $A_1$, ~~is present~~ or ~~by~~ when a mutant ~~derived from~~ $a_1^{m-1}$ arises whose action resembles that of $A_1$ ~~that resemble $A_1$ in their action.~~

In addition to the 2 cases of modified $a_1^{m-1}$ behavior, ~~action that result in the appearance of phenotypes~~ described above, tests were conducted with ~~30~~ 28 other plants grown in the greenhouse during the winter of 1950-51. Four of them (Row 1, table 1) were derived from kernels that appeared to be uniformly pigmented and the type ~~bein~~ was similar to that produced ~~by~~ when standard $A_1$ is present. In the progeny derived by test crosses of three of them, the ~~stabel~~ $A_1$ phenotype appeared in expected proportions and there was no evidence of instability in expression. The fourth ~~proved to have~~ progeny of this plant indicated the presence of a modified $a_1^{m-1}$. Kernels carrying it exhibited ~~behavior in that it produced~~ very many ~~mutations that occurred~~ mutant areas that arose from mutations that had occurred late in development. It is probable, therefore, that the phenotype of the kernel that gave rise to this plant was produced ~~by~~ because of confluence of haloes about mutant areas in the manner ~~as~~ described above. Five other plants (Row 2, table 1), each derived from a kernel exhibiting only pale pigmentation, uniformly distributed over the aleuron layer, were also examined. The pale phenotype ~~appeared to be quite stable in expression and it~~ was recovered in expected proportion in ~~the~~ progeny derived from tests crosses made with each of these plants. No variegated kernels appeared.

Tests of variegated plants

In addition, tests were conducted with 19 other plants, each of which arose ~~derived~~ from a variegated kernel. Five (Row 4, table 1) came from kernels that had exhibited the pattern of this that was common to the majority of variegated kernels on ears produced by test crosses conducted with the original $a_1^{m-1}$ carrying plant. Each of the remaining 14 plants came from a kernel that had exhibited some marked deviation from ~~of~~ this pattern, and the types of this are described in rows 5 to 10 of column 1 of table 1. In this table, the culture number~~s~~ of each plant is given, and that of four of them are ~~has been~~ underlined. From them ~~These four plants are the source of~~ originated four of the six modified states of $a_1^{m-1}$ that were used extensively throughout the study of $a_1^{m-1}$. In subsequent references to a particular state, it will be designated by the culture number of the plant that ~~which~~ originally carried it. It may be mentioned ~~stated~~ at this time that although the original state of $a_1^{m-1}$ has given rise to many altered ~~different~~ states, only a 16 of them ~~small representative sample of types~~ ha~~d~~s been ~~received full~~ examined ~~examination~~.

No clear indication ~~evidence~~ of a two-element system, responsible for control of gene expression at $a_1^{m-1}$, was obtained from ratios of kernel types on ears produced by self-pollination or by test cross of the plants grown in the greenhouse during the winter of 1950-51. ~~Several examples will be given which will illustrate this. Plant 5715A, row 4, table 1, arose from a kernel that had very many small deeply pigmented spots xxxxxx in a colorless background.~~

Examples illustrating this are given in table 2. In this table, the phenotypes of kernels produced by tests crosses conducted with the ~~6~~ six plants entered in line ~~4 or 5~~ 7 of table 1 are ~~entered~~ given. All ~~of the~~ six variegated plants were $a_1^{m-1}/a_1$, $A_2/A_2$ in constitution. In ~~this~~ table 2, the progeny kernels derived from ~~from~~ any one test ~~is~~ are divided into three classes; ~~the~~ uniformly pigmented, ~~kernels the~~ variegated, ~~kernels~~ and ~~the~~ colorless. ~~kernels~~ The intensity of pigment exhibited by ~~of~~ kernels entered in the uniformly pigmented class differed according to the plant that was tested. Those derived from tests of plant 5715A were intensely pigmented. The ~~intensity of~~ pigment in those derived from plants 5717A, 5719A-1, and 5719A-2 ~~were intense but less so~~ was deep but it was slightly less intense. In contrast, the ~~intensity of~~ kernels in this class in the progeny of plants 5714E and 5718 expressed a very low level of intensity ~~of~~ pigment. ~~They were~~ All were very pale.

[illegible] in progeny of the plant

Similarly, differences were expressed in the pattern of variegation exhibited by kernels in the variegated class in the progeny of plants entered in row 7 of table 1. In the progeny of plant 5715A, the~~se~~ variegated kernels were characterized by the presence of very many, small, deeply-pigmented spots. ~~In a~~ A few of these kernels also had a large, deeply-pigmented area. ~~was produced~~. The pattern given by the variegated class of kernels in the progeny of plants 5719A-1 and 5719A-2 ~~were~~ was alike, and it resembled that given by plant 5715A except that there were far fewer deeply pigmented spots. Plants 5717A and 5718 produced variegated kernels in their progeny that had small, deeply pigmented spots but the numbers of them were even fewer than those in the variegated class of kernels in the progeny of ~~produced by~~ plants 5719A-1 and 5719A-2, ~~and~~ also no large pigmented areas appeared in any of them. The pattern exhibited by the variegated kernels in the progeny of plant 5714E was likewise distinctive. ~~Only small~~ The spots of deep pigmentation were very small ~~appeared in them~~ and these were ~~distributed~~ widely dispersed ~~lightly~~ over the aleurone layer.

Tests subsequently conduced with the progeny of the plants entered in table 2,-- and that of plant 5718 and 5719A-1 ~~some of them~~ through many plant generations-- indicated that the pattern of variegation given by each was a reflection of some modification that had occurred to $a_1^{m-1}$ in a cell of the original $a_1^{m-1}$ carrying plant. This modification was responsible for the altered pattern of expression of $a_1^{m-1}$ given by each of the plants entered in table 2. It is known that this expression will persist through ~~cell and~~ plant generations until another event occurs in some cell of a plant that again ~~to~~ modifies the behavior of $a_1^{m-1}$ and this modified behavior will be expressed in ~~subsequent~~ descendant cells~~, and plant generations~~. That the degree of stability of any one state of $a_1^{m-1}$ through plant generations ~~is related to~~ depends, in part, on the time of occurrence of mutation-inducing events, -- those events that produced the deeply pigmented spots in the progeny tests just described,--will be pointed out ~~shortly~~ later. It need only be stated here that the later the time during development that such mutations occur, the less the change of appearance in a gamete of a newly altered state, and a /~~N~~ewly altered state~~s~~ must be present in a gamete~~s~~ if it is to be isolated for further examination.

Plant 5720, row 9, table 1, arose from a kernel that exhibited both large and small pigmented areas in a colorless background but the intensity of pigment in nearly all of them was much lower than that produced by $A_1$. The different areas exhibited pigment of quite different intensities, ranging from very pale in some to quite dark ~~intense~~ in others. The plant also exhibited pigmented areas ~~variegation~~ in which different grades of intensity were expressed. Progeny of this plant, derived from self-pollination and from reciprocal crosses with plants homozygous for $a_1$ ~~and $a_2$~~, produced variegated kernels of the same type as that which gave rise to this plant. In a few ~~some~~ of them, however, one or several very small spots of the full $A_1$-type pigment appeared. In addition, there were many kernels exhibiting pigment of one grade of intensity uniformly distributed ~~pigment~~ over all of the aleurone layer, ~~and~~ the intensity of pigment ~~of this~~ ranged from very light in some kernels to quite dark in others. ~~These kernels expressed~~ Among them the same grades of pigment intensities were expressed as that exhibited among/the different/pigmented areas in a ~~the~~ variegated kernel~~s~~. There were also a number of colorless kernels. Subsequent study of the progeny of plant 5720 made it evident that a modification of $a_1^{m-1}$ had occurred in a sporogenous or spore cell of the original $a_1^{m-1}$ carrying/plant that had parent effected a marked shift in the proportions of different mutant phenotypes it would produce in comparison with those given by the original $a_1^{m-1}$ state. However, this alteration did not ~~alter~~ modify the time during development at which a mutation-inducing event would occur, as it had done ~~xxxxxx xxxxx xxxxxxxxxxxxxx~~ with respect to ~~the~~ $a_1^{m-1}$ carried in the plants entered in ~~line~~ row 7, table 1, described above.

As mentioned earlier, none of the tests conducted with plants grown in the greenhouse during the winter of 1950-51 ~~indicated~~ gave evidence suggesting the presence of an independently located element that was associated with control expression of gene ~~instability~~ ~~of~~ $a_1^{m-1}$. Only ~~when~~ after progeny of some of these greenhouse plants were ~~grown~~ examined and test-crossed during the summer of 1951 was ~~it realized~~ evidence of this obtained ~~that an independently located element was associated with this~~, and the type ~~of evidence of this~~ will be considered below.

Discovery of an independently located and controlling element in the $a_1^{m-1}$ ...
~~2. Tests of the constancy of behavior of different states of $a_1^{m-1}$~~

During the summer of 1951, ~~plants were grown from~~ selected kernels in the progeny ears ~~derived from~~ produced by crosses conducted with five of the plants entered in table 1, 5700A, 5718, 5719A-1, 5719A-2, and 5720 were grown. In addition, plants were grown from kernels with special phenotypes that were selected from ears produced by cross of the original $a_1^{m-1}$ carrying plant (5381) to plants that were homozygous for $a_1$. ~~Among the latter, there were 48 plants derived from~~

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kernels exhibiting the pattern of variegation appearing in most of the kernels receiving $a_1^{m-1}$ from the parent plant, 4 plants derived from kernels whose variegated patterns deviated markedly from this, 7 plants derived from kernels that appeared to be fully $A_1$ in phenotype, and 7 other plants derived from kernels that were uniformly but less intensely pigmented.

A record was made of the phenotype exhibited by each plant and test crosses were conducted with each. many of these plants were crossed ~~An effort was made to cross each plant~~ with ones that were homozygous for the standard recessive, $a_1$, and for the recessive, $sh_2$ (shrunken endosperm) which is very closely linked to $a_1$, giving only 0.4 percent recombination with it. This stock had been obtained from Dr. M.M.Rhoades for the purpose of making ~~a~~ more accurate examinations of ~~the~~ different independently arising modifications of gene action ~~types of behavior of instability~~ of $A_1$. ~~Independently~~ ~~arising~~ Cases of this had been accumulating in my cultures. Close linkage of a well expressed gene ~~tic~~ marker with ~~the~~ an ~~unst~~able locus would allow accurate ~~detection~~ determinations ~~identification~~ of some types of ~~genetic~~ change in ~~gene~~ its action ~~of the~~ ~~unstable locus~~ that otherwise might not be readily detected. Since all $a_1^{m-1}$ plants grown in the summer of 1951 were homozygous for $Sh_2$, crosses of them with plants homozygous for $a_1$ and $sh_2$, prepared the way for more precise investigations of ~~it~~ $a_1^{m-1}$ in the future. ~~Other tests of these plants~~

In addition,

~~also were made.~~ Some plants were self-pollinated, ~~and~~ also crossed ~~to~~ were made between

plants carrying ~~other~~ unlike states of $a_1^{m-1}$, ~~or to those derived from the pale~~

~~class of kernels.~~ ~~However,~~ At this time, other projects were in progress

that took precidence over ~~the immediate continued~~ study of $a_1^{m-1}$ and

made in the summer of 1951

therefore, the ears obtained from the various types of cross were not

carefully examined until more than a year had elapsed. Included in these

other projects was examination of the behavior of $a_1^{m-2}$, $a_1^{m-3}$, and $a_1^{m-4}$,

three independent inceptions of instability ~~xxxxxxxxx~~ of gene expression

at the locus of $A_1$. It was soon realized that ~~instability~~ ~~control of~~

was controlled by

gene expression at $a_1^{m-3}$ and $a_1^{m-4}$ ~~resided in~~ the Ds-Ac system ~~of control~~

~~of gene action.~~ The system controlling $a_1^{m-2}$ was not Ds-Ac, nor that

in which Dt is involved. A system having ~~with~~ a quite different mode of operation ~~action~~

was responsible for its ~~types of~~ expression. An interpretation of the

mode of operation of this system was formulated from tests conducted with

it during the ~~over a period of~~ preceding three years. Evidence obtained from ~~From results of~~ tests / crosses conducted ~~obtained during~~

with them ~~the summer of 1952~~ with ~~the~~ few A- plants carrying $a_1^{m-1}$ were grown in the summer of 1952 ~~plants that were grown and also~~

~~from examination of kernel types of ears produced during the previous~~

suggested to control gene action

~~summer,~~ ~~it was suspected~~ that the system operating at ~~both~~ $a_1^{m-1}$ ~~and $a_1^{m-2}$~~

(similar to that effecting control of gene action at $a_1^{m-2}$ study of $a_1^{m-1}$

might be ~~the same~~. Subsequent ~~tests~~ indicated, however, that this

assumption was incorrect. The two systems do not have elements in common nor is their mode of operation comparable.

Even though a decision had been made to postpone study of $a_1^{m-1}$ until more time would be available for its examination, it was desired to learn if the change at $A_1$, responsible for the origin of $a_1^{m-1}$, would effect an alter ation in crossing-over between this modified locus and $sh_2$. To determine this, only a few plants would be required and only a small amount of time would need to be given to ~~accomplish~~ this test. Therefore, 10 variegated kernels were removed from an ear produced by each of two variegated plants that were $a_1^{m-1}$ $Sh_2/a_1$ $Sh_2$ in constitution when pollen from a plant homozygous for $a_1$ and $sh_2$ had been ~~was~~ used on the silks of each ear. ~~Both~~ Each of the two variegated plants had been derived from a variegated kernel on the ear produced by the cross conducted with plant 5719A-1, entered in C of table 2. therefore both of them had the 5719A-1 state of $a_1^{m-1}$. Nineteen mature plants were obtained from the 20 selected kernels. All exhibited variegation for anthocyanin pigment, and all were $a_1^{m-1}$ $Sh_2/a_1$ $sh_2$ in constitution. The silks of all fertile ears produced by 17 of these plants received pollen from plants that were homozygous for $a_1$ and for $sh_2$. The reciprocal cross was conducted with 8 of these variegated plants. The kernel types appearing on the ears these crosses produced are entered in table 3.

the distribution of phenotypes within the Sh2 and sh2 classes indicate

In table 3, (very close linkage ~~is expressed~~ between Sh2 and the ~~classes of kernels that show pigment in them~~ locus responsible for the appearance of anthocyanin pigment. Within the 7490 kernels that exhibited pigment, there were three distinct classes. In two of them, pigment was uniformly distributed over the aleurone layer. Of the 3617 kernels with such uniformly distributed ~~of~~ pigment, only 4 ~~xxxx~~ had the type of pigment produced when $A_1$ is present. The pigment in the other ~~xxxxx~~ 3613 kernels was clearly not the same; they are entered in this table under the heading of "pale". ~~distinctly different~~, and this was well expressed by the ~~distinctions~~ difference ~~xxxxxxxxxxxxxxxxxx xxxxxxxxxxxxxxxxxxxxxxxxx~~ in pigment intensity ~~between~~ shown by ~~in~~ those that had the genetic marker Pr when compared with ~~and~~ those that were homozygous for the recessive allele, pr. With the standard $A_1$, the presence of Pr results in the production of intensely purple pigment~~s~~. When pr is homozygous, the kernels exhibit, with $A_1$, an intense deep-red color. Some of the $a_1^{m-1}$ plants had Pr in one chromosome 5 and pr in the homologue. The $a_1, sh_2$ tester plants were homozygous for pr. Therefore, on some of the ears, half of the kernels carried Pr and the other half was homozygous for pr. On these ears, it was very evident that the intensity of pigment appearing in a kernel in the uniformly ~~pale~~ pigmented class ~~of kernels~~ (the pale class in Table 3) depended upon ~~their~~ its constitution with respect to the alleles of Pr. The color in those kernels having Pr was a deep purple, almost as intense as that produced when the standard $A_1$ is present. ~~On the other hand, those kernels that were homozygous for pr were only~~

In contrast, those homozygous for pr were a very light shade of pink. It was apparent from this that the pigment present in the pale class of kernels did not ~~represent~~ suggest dilution of that produced by $A_1$, but rather ~~a distinctive~~ the presence of a different type of pigment. ~~All of the pale kernels that were Pr~~ The Pr kernels entered in the pale class were alike among themselves in phenotypic expression and, ~~xxxx~~ all those that were pr were ~~xxxxxxx~~ likewise alike. The pale class represents, then, one particular genotype. Among the variegated class of kernels, the pigment type in the ~~xxxxxxxx~~ deeply pigmented spots resembled that produced by $A_1$, either a very deep purple or a very deep red depending on the presence or absence of Pr in them. (Pr, purple aleurone, pr recessive allele, red aleurone, located in chromosome 5.) The pattern of such spots was quite similar in all but a very few kernels. [See page 00 for a description of the pattern of variegation exhibited by these few exceptional kernels]. Illustrations of this pattern are given in photo .

In examining the data of table 3, attention should be given to the ratio of the pale class to the variegated class appearing on different ears ~~produced by each~~ of a single plant, and on these ears derived from use of its pollen in the test cross to plants homozygous for $a_1$ and $sh_2$. It may be seen that an approximate ratio of 1 pale to 1 variegated kernel appeared on ears produced by tests crosses conducted with plants 1, 2, 4, 5, 6, 7, and 8 in culture 6452 and by plants 1, 2, 4, 5, 6, 7, and 8 in culture 6453, although there are some marked deviations from this expressed by tests of the pollen of plants 6452-5 and 6453-4. The ratio of pale to variegated kernels derived from